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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

ROBERT T. FRALEY and
STEPHEN G. ROGERS

SERIAL NUMBER: 07/625,637

FILED: December 7, 1990

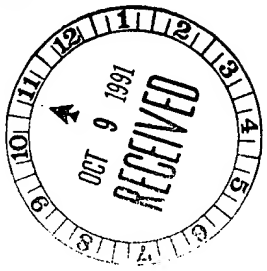
TITLE: CHIMERIC GENES FOR TRANSFORMING PLANT CELLS USING VIRAL PROMOTERS

GROUP ART UNIT: 184

EXAMINER: D. Fox

September 26, 1991

Considered
12/15/91
DF



DECLARATION under 37 C.F.R. § 1.132

Commissioner of Patents and Trademarks

Washington, D. C. 20231

Sir:

I, Charles L. Armstrong, declare that I am a United States citizen and a resident of Creve Coeur, Missouri. I further declare that I am a plant biologist by profession having graduated with a Doctor of Philosophy degree in Plant Breeding from the University of Minnesota in 1986. I further declare that from November, 1985 to September, 1988 I held the position of Research Scientist in the laboratory of Agrigenetics Company at Madison, Wisconsin and since October, 1988 to present I have been employed by Monsanto Company at St. Louis, Missouri as a Research Specialist in plant molecular biology research. I have extensive experience in the evaluation of expression vectors for use in plants and the function of promoters of chimeric genes to produce heterologous proteins in plants. I am the author or co-author of five (5) articles involving plant molecular biology published in scientific journals.

I am familiar with the above identified patent application. It is my understanding that this application describes the identification and isolation of cauliflower mosaic virus (CaMV) promoters, the construction of plant expression vectors comprising a chimeric gene containing the CaMV promoter, and the

evaluation of the expression of said chimeric gene to produce heterologous protein in plants.

I am familiar with U.S. Patent No. 4,536,475 issued to Anderson on August 20, 1985. This patent describes the construction of a chimeric gene containing a Herpes Simplex virus thymidine kinase promoter which gene is alleged to confer kanamycin resistance when expressed in plant cells.

In my experience, research and study of the technical literature, the results reported in the Anderson patent have not been duplicated or confirmed by other investigators. Furthermore, I have no knowledge of any reference that teaches the use of a non-plant promoter to cause the expression in plants of a chimeric gene. It is generally accepted in the plant biology field that a non-plant promoter will not function in plants and that only plant promoters will function at measurable levels in plant cells.

I have read and understand the Declaration of Dr. Robert B. Horsch submitted with the Preliminary Amendment in this application. This declaration describes experiments in which plant expression vectors were constructed comprising CaMV promoter of the subject invention driving a structural DNA sequence encoding for neomycin phosphotransferase which enzyme imparts kanamycin resistance. Control plant expression vectors were constructed in which the Herpes Simplex thymidine kinase promoter of Anderson replaced the CaMV promoter. These vectors were used to transform tobacco cells and the transformed cells were grown on media containing kanamycin. Under this regimen, only transformed cells expressing neomycin phosphotransferase will grow. The experimental protocols and evaluations followed conventional scientific practice. The data unequivocally demonstrated that the cells transformed with CaMV expression vector exhibited significant kanamycin resistance which permits selection between transformed cells and untransformed

cells, whereas, the cells transformed with Anderson Herpes Simplex thymidine kinase expression vector exhibited insufficient kanamycin resistance to permit selection between transformed cells and untransformed cells. In fact, the data shows no difference in kanamycin resistance between plant cells transformed using the Anderson expression vector and transformed control plant cells lacking the KAN gene.

I have examined the data of Figure 3 and disagree with the assertion of the Patent Examiner that data for pMON16301 containing the thymidine kinase KAN gene exhibits evidence of increased callus growth and shoot production. On the contrary, the evidence shows no difference between the negative control and the thymidine kinase KAN gene. This is brought out in the Horsch declaration which states on page 6 that kanamycin at 300 μg/ml almost completely inhibits growth of callus and shoots of the transformed tobacco cells containing no KAN gene. The experiment with plant cells transformed with the tk/KAN vector gave the same results, the declaration states said plant cells exhibited "almost complete inhibition of growth of the transformed tobacco cells." This describes the same results as obtained with the control.

Similar results were obtained in experiments where the callus was grown on kanamycin at 100 μg/ml. The declaration on page 6 states:

> "Negative control leaf discs transformed with a vector lacking a KAN gene produce some limited growth of callus and shoots. Similarly, leaf discs transformed with the tk/KAN gene also produce some limited growth of callus and shoots at this level of kanamycin. The amount of growth of callus and shoots in the tk/KAN containing leaf discs is very similar to the amount of growth exhibited in the negative control leaf discs." (emphasis added)

This clearly means that there is no distinguishable difference between the negative control and the tk/KAN plant cells. This data indicates that the tk/KAN plant cells express no neomycin phosphotransferase or an insignificantly low

amount insufficient to impart sufficient kanamycin resistance to permit growth on kanamycin containing growth media. These cells performed the same and provide no evidence whatsoever of increased callus growth and shoot production as asserted in the Patent Office Letter.

The data of the Horsch declaration shows that expression vectors containing the CaMV promoter of the invention exhibit significantly higher expression than expression vectors containing the tk promoter. The superior expression level with the CaMV promoter was not predictable from the teaching of the Anderson patent. The level of expression obtained from the tk promoter is so low to be regarded as worthless since it is inadequate to select transformed cells from untransformed cells, the very purpose for imparting kanamycin resistance to transformed plant cells.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted,

Charles L Armstrong

Charles L. Armstrong

Date: 9/26/91